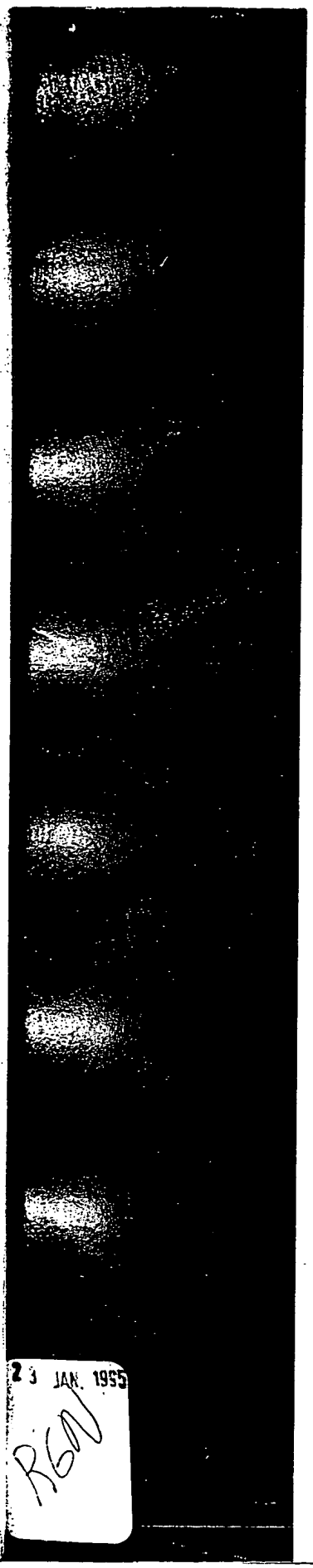

American Heart Association℠
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Circulation Research

Volume 76, Number 1 January 1995

Original Contributions

Brief Definitive Communication

Rapid Communications

73-5133(SP) ISSN 0009-7330

Enhanced Preservation of Orthotopically Transplanted Rat Lungs by Nitroglycerin but Not Hydralazine

Requirement for Graft Vascular Homeostasis Beyond Harvest Vasodilation

Yoshifumi Naka, Nepal C. Chowdhury, Hui Liao, Dilip K. Roy, Mehmet C. Oz, Robert E. Michler, David J. Pinsky

Abstract Nitric oxide (NO) produced within the lungs maintains pulmonary vascular homeostatic properties, modulating leukocyte traffic, platelet aggregation, and vasomotor tone. Because reactive oxygen intermediates generated during reperfusion react rapidly with available NO, we hypothesized that the NO donor nitroglycerin (NTG) would enhance lung preservation for transplantation by improving graft blood flow and reducing graft neutrophil and platelet sequestration. By use of an orthotopic rat left lung transplant model, with ligation of the native right pulmonary artery to ensure that recipient survival and physiological measurements depend entirely on the transplanted lung, transplants were performed in 70 male Lewis rats after 6-hour 4°C preservation in Euro-Collins solution (EC) alone or EC with supplemental NTG. Compared with EC alone, supplemental NTG significantly increased pulmonary arterial flow (2.2±1.4 to 21.4±2.9 mL/min, $P<.01$), decreased pulmonary vascular resistance (7.4±2.0 to 1.4±0.1×10³ Woods units, $P<.05$), improved arterial oxygenation (163±57 to 501±31 mm Hg, $P<.01$), and enhanced recipient survival (17% to 100%, $P<.001$). These beneficial effects of NTG were dose dependent over a range of 0.001 to 0.1 mg/mL. Although NTG caused significant pulmonary vasodilation during the harvest/flushing period, the direct-acting vasodilator hydralazine caused greater vasodilation than did NTG but was associated with poor graft function, elevated pulmonary vascular resistance, and poor recipient survival. To explore nonvasodilator protective mechanisms of NTG, graft neutrophil and platelet sequestration were studied; supplemental NTG significantly reduced both neutrophil and platelet accumulation compared with either hydralazine or EC alone. These findings suggest that vasodilation alone at the time of harvest is insufficient to protect the lungs. NTG, which produces antineutrophil and antiplatelet effects as well as harvest vasodilation, appears to be a simple and effective additive that will improve lung preservation for transplantation. (*Circ Res.* 1995;76:900-906.)

Key Words • nitroglycerin • hydralazine • lung transplantation • harvest vasodilation

There has been a recent burgeoning of clinical lung transplantation,[1] prompted in part by the development of improved pulmonary preservation solutions, but the lungs remain among the organs most vulnerable to ischemia and reperfusion injury during the transplantation process. The inability to preserve lungs beyond 4 to 6 hours is a major impediment for immunologic cross-matching and hampers efforts at multiple or distant organ procurement. Even with optimal preservation techniques, the perioperative morbidity and mortality remain high, with early graft failure characterized by elevated pulmonary vascular resistance (PVR), poor gas exchange, and neutrophil infiltration.[2] Recent studies have demonstrated that maintaining endothelial function within cardiac grafts is critical to successful cardiac preservation,[3-5] but the current gold-standard clinical lung preservation solution, modified Euro-Collins solution (EC), consists of a simple electrolyte solution without additives to specifically address the maintenance of endothelial function.[2] Because the lungs are among the most richly vascularized of organs, we hypothesized that maintaining normal endothelial properties during preservation and transplantation of the lungs would be critical to the ultimate success of lung transplantation, especially after periods of prolonged preservation.

Of the numerous factors that influence vascular function, endothelium-derived relaxing factor (EDRF, whose identity appears to be nitric oxide [NO][6-8]) has emerged as a key modulator of normal pulmonary vascular physiology. In addition to preventing neutrophil adherence to the endothelium,[9] maintaining endothelial barrier properties,[10] and inhibiting platelet aggregation,[5] NO has an important role in modulating pulmonary vascular tone. Endogenous pulmonary NO production participates in the physiological regulation of pulmonary vasomotor tone, as has been shown in animal models by the use of inhibitors of NO synthase,[11-15] although the degree of importance is affected by species and experimental conditions under which observations are made. Even in humans, NO can be identified in exhaled air[16] and is thought to regulate basal PVR.[17] Models of cardiac ischemia and reperfusion have demonstrated that both EDRF bioactivity[18] and NO levels[5] fall within minutes of reperfusion because of the quenching of

Received August 10, 1995; accepted January 23, 1995.

From the Departments of Physiology (Y.N., H.L.), Surgery (N.C.C., D.K.R., M.C.O., R.E.M.), and Medicine (D.J.P.), Columbia University College of Physicians and Surgeons, New York, NY.

Correspondence to David J. Pinsky, MD, Department of Medicine, Columbia University College of Physicians and Surgeons, 630 West 168th St, New York, NY 10032.

NO by superoxide generated during reperfusion; this reaction is rapid, with a rate constant of 10^8 $(mol/L)^{-1} \cdot s^{-1}$, forming peroxynitrite in the process.[19] Because reactive oxygen intermediates are formed in especial abundance in the pulmonary reperfusion microenvironment,[2,20] we hypothesized that endothelium-dependent vascular homeostatic properties might be perturbed by the lack of available NO and that pulmonary preservation might be enhanced by nitroglycerin (NTG), an NO donor. In this study, we used a recently developed model of rat orthotopic lung transplantation in which the native right lung supports the animal during surgery but is effectively removed from the circulation after transplantation so that physiological measurements and recipient survival represent function of the transplanted lung.[21] Experiments were designed to test (1) whether vasodilation at the time of harvest is sufficient to improve lung preservation and (2) whether NTG supplementation would enhance NO-related mechanisms of vascular homeostasis after lung transplantation.

Materials and Methods

Orthotopic Left Lung Transplantation

By use of inbred male Lewis rats (250 to 300 g), hemodynamic and oxygenation measurements were made in an orthotopic left lung transplant model.[21] In brief, the donor rat was heparinized (500 U IV), and the superior venae cavae were ligated. Thirty milliliters of 4°C preservation solution was infused into the inferior vena cava at a constant pressure (20 mm Hg) and vented out the left atrium (LA). The time required to deliver the 30-mL volume of preservation solution at constant infusion pressure was recorded as an index of PVR during harvest. The left pulmonary artery (PA) and pulmonary vein (PV) were then divided, the bronchus was ligated and divided with the lung partially inflated, and the lung was removed. A 14-gauge cuff was placed on each vascular stump, a 16-gauge grooved cylinder was inserted into the bronchus, and the lung was submerged in 4°C preservation solution for 6 hours. The recipient rat was anesthetized and intubated (ventilated with 100% oxygen); a left thoracotomy was performed; the left bronchus, PA, and PV were isolated, cross-clamped, and divided; and the native lung was removed. The cylinder (bronchus) and cuffs (PA and PV) were connected to the appropriate recipient structures, maintaining warm ischemic times below 10 minutes. The hilar cross clamp was released, reestablishing blood flow and enabling gas exchange. A snare was then passed around the right PA, and 2F catheters (Millar instruments) were introduced into the main PA and the LA. A flow probe (Transonic) was then placed around the main PA.

Preservation solutions consisted of modified EC, EC with supplemental NTG (5 mg/mL, intravenous formulation, DuPont Merck Pharmaceuticals), or EC with supplemental hydralazine (CIBA-GEIGY Limited). EC solution was purchased from Baxter Healthcare and consisted of Na^+ (10 mEq/L), K^+ (115 mEq/L), Cl^- (15 mEq/L), HPO_4^{-2} (85 mEq/L), $H_2PO_4^-$ (15 mEq/L), and HCO_3^- (10 mEq/L), modified by adding magnesium sulfate (10 mL of 10% solution) and glucose (50 mL of 50% solution) to each liter before use. After flushing the lungs with hypothermic preservation solutions as described, harvested lungs were preserved for 6 hours at 4°C in 50 mL of preservation solution with a composition identical to that used during harvest.

Measurement of Lung Graft Function

On-line hemodynamic monitoring was accomplished by using a MacLab and a Macintosh IIci computer. The hemodynamic parameters that were measured included LA pressure (in millimeters of mercury), PA pressure (in millimeters of mercury), and PA blood flow (in milliliters per minute). Arterial oxygen tension (in millimeters of mercury) was measured during inspiration of 100% oxygen; Po_2 was analyzed with a model ABL-2 gas analyzer (Radiometer). PVRs were calculated as follows: (mean PA pressure−LA pressure)/PA flow, expressed as Woods units$\times 10^3$. After baseline measurements were taken, the native (right) PA was ligated, and serial measurements were taken every 5 minutes until the time of euthanasia at 30 minutes (or until recipient death, if it preceded the 30-minute time point). Recipient death was identified by cessation of cardiac mechanical activity as viewed through the open thorax. To determine whether longer survival was possible in lungs preserved with supplemental NTG (0.1 mg/mL), for certain experiments, observation was continued until 8 hours after surgery without hemodynamic measurements, and death was identified as described above.

Myeloperoxidase Assay

Thirty minutes after ligation of the native right PA or at the time of recipient death, transplanted lungs were removed, rinsed briskly in physiological saline, and snap-frozen in liquid nitrogen until the time of myeloperoxidase assay.

Tissue was homogenized in phosphate buffer (50 mmol/L, pH 5.5, 5 mL/g of tissue) containing hexadecyltrimethylammonium bromide (0.5%, Sigma Chemical Co). The assay was performed, as previously described,[22] by thawing the sample, centrifuging at 40 000g for 15 minutes, and decanting the supernatant, which was assayed for myeloperoxidase activity by using a standard chromogenic spectrophotometric technique in which test sample (0.03 mL) was added to phosphate buffer (0.97 mL) containing *o*-dianisidine dihydrochloride (Sigma) and hydrogen peroxide (0.0005%) and change in absorbance at 460 nm was measured over 1 minute (increase in optical density was linear over this time interval).

Measurement of Graft Platelet Accumulation and Bleeding Time

Graft platelet accumulation was determined by using ^{111}In-labeled platelets, prepared as previously described.[23] Blood (5.0 mL) was taken from a gender/strain-matched donor rat and heparinized (2500 U). Platelets were isolated by differential centrifugation, first at 300g for 5 minutes to obtain platelet-rich plasma, which was then washed three times at 2000g for 15 minutes in 10 mL of acid/citrate/dextrose anticoagulant (ACD-A, containing 38 mmol/L citric acid, 75 mmol/L sodium citrate, and 135 mmol/L glucose). The pellet was suspended in 5 mL of ACD-A and centrifuged at 100g for 5 minutes to remove contaminating red blood cells, and the supernatant was collected. ^{111}In oxyquinoline (70 μL of 1 mCi/mL, Amersham Mediphysics) was added with gentle shaking for 30 minutes at room temperature. The radiolabeled platelets were washed three times in ACD-A and resuspended in PBS, and platelet number was adjusted to 5×10^7/mL. After completion of the vascular and bronchial anastomoses, 0.5 mL of ^{111}In-labeled platelet suspension was injected intravenously into the recipient. One minute after platelet infusion (immediately before reperfusion), 0.5 mL of blood was taken from the LA to determine blood radioactivity to ascertain blood platelet concentrations to normalize for variations in blood loss during surgery. Five minutes after reperfusion, the native right PA was ligated. The graft was removed 10 minutes thereafter, and ^{111}In-labeled platelet deposition was quantified by gamma counting. Platelet accumulation was expressed as the ratio of graft radioactivity to blood radioactivity normalized to dry weight. Platelet function was measured by evaluating bleeding time in pulmonary transplant recipients as previously described[24]; immediately after reperfusion, the rat tail was transected 5 mm proximal to the tip, and every 30 seconds, blood drops were collected onto a piece of filter paper placed tangentially to the tail. Bleeding time was recorded as the time from the initial transection to the cessation of bleeding.

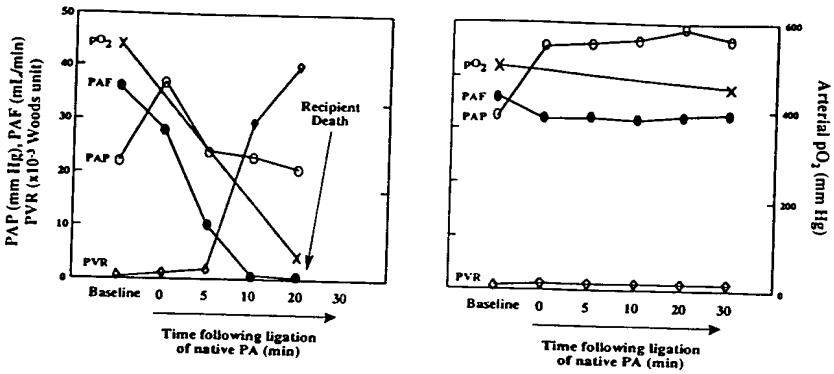

FIG 1. Graphs showing representative hemodynamic tracings of a lung transplant after hypothermic preservation for 6 hours in Euro-Collins solution (EC) alone (control, A) or EC supplemented with nitroglycerin (0.1 mg/mL, B) after the native lung was removed from the pulmonary circulation, as described in text. PAP indicates mean pulmonary arterial pressure; PAF, pulmonary arterial flow; PVR, pulmonary vascular resistance; and PA, pulmonary artery.

Statistics

Recipient survival data were evaluated by using Fisher's exact test. All other data were evaluated by using the Mann-Whitney *U* test. Because survival assessment was measured independent of hemodynamic measurements, all survival data are included in "Results," even when equipment malfunction precluded obtaining hemodynamic measurements. Values are expressed as mean±SEM, with differences considered statistically significant at $P<.05$.

Results

To determine whether supplementation of the clinical standard pulmonary preservation solution with NTG would enhance pulmonary vascular function and improve recipient survival after lung transplantation, experiments were performed by using EC as the base solution. A preservation duration of 6 hours was chosen for these experiments on the basis of pilot studies demonstrating significant graft failure with EC alone at this preservation duration[21] and the clinical relevance of 4 hours as the upper limit of acceptable pulmonary preservation in humans. At 6 hours of preservation in EC alone, graft failure occurred rapidly after pulmonary reperfusion, with marked increases in PVR accompanied by declines in PA flow and arterial oxygenation (Fig 1A). Although PA pressure rose initially upon ligation of the native (right) PA, this was followed by a rapid decline in PA pressure, followed by recipient death. In sharp contrast, when NTG was added to the preservation solution, hemodynamic and functional (arterial oxygenation) parameters were stabilized, and the recipient survived the 30-minute observation period (Fig 1B). Compared with the control preservation solution (EC alone), NTG (0.1 mg/mL) added to the preservation solution increased PA flow (2.2±1.4 versus 21.4±2.9 mL/min, $P<.01$) (Fig 2A), decreased PVR (7.4±2.0 versus 1.4±0.1×10³ Woods units, $P<.05$) (Fig 2B), and improved arterial oxygenation (163±57 versus 501±31 mm Hg, $P<.01$) (Fig 2C).

To ensure that these beneficial effects of NTG were due to NTG itself rather than the diluent found in the intravenous formulation that we used to prepare our preservation solution, EC was supplemented with an equivalent amount of diluent, and the effects on graft preservation were measured (n=3); for these experiments, preservation was not different from that found with the control solution (PVR was 6.1±3.3×10³ Woods units, and arterial oxygenation was 127±47 mm Hg; P=NS versus control solution).

Recipient survival was also improved significantly by supplementation of the preservation solution with NTG compared with the control solution (17% versus 100%, respectively; $P<.001$). The beneficial effects of NTG were dose dependent over a range of 0.001 to 0.1 mg/mL, with maximal beneficial effect obtained at 0.1 mg/mL (Fig 3). To determine whether recipients of grafts preserved with supplemental NTG (0.1 mg/mL) could survive beyond the 30-minute time point, at which

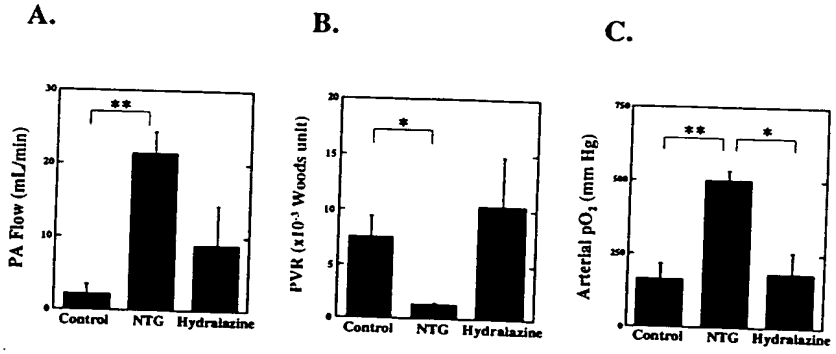

FIG 2. Bar graphs showing the effects of nitroglycerin (NTG) or hydralazine on lung preservation for transplantation. All lung transplants were performed after 6 hours of hypothermic preservation in Euro-Collins solution alone (control, n=6 for panels A and B) or Euro-Collins solution supplemented with NTG (0.1 mg/mL, n=5) or hydralazine (0.02 μg/mL, n=6). Measurements were recorded at the final time at which the recipient was alive or at 30 minutes after ligation of the native right pulmonary artery (PA). A shows PA flow; B, pulmonary vascular resistance (PVR); and C, arterial oxygenation (n=10 for control, including oxygenation data from four pilot experiments in which hemodynamics were not measured). Values are mean±SEM. *$P<.05$ and **$P<.01$.

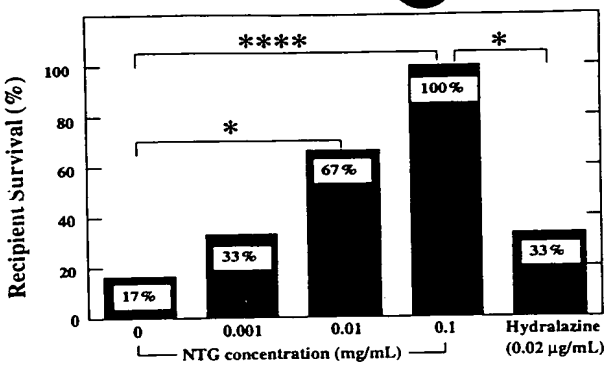

FIG 3. Bar graph showing dose-dependent effects on recipient survival elicited by nitroglycerin (NTG) compared with hydralazine. Experiments were performed as described in Fig 2. The dose-response relation of NTG showed maximal ability to enhance lung preservation at 0.1 mg/mL (n=18 at 0 mg/mL, n=3 at 0.001 mg/mL, n=3 at 0.01 mg/mL, and n=7 at 0.1 mg/mL). *$P<.05$ and ****$P<.001$.

we routinely assess survival and graft hemodynamics, six additional experiments were performed in which recipients were observed after the transplantation procedure for up to 8 hours after transplantation and ligation of the native PA. All six recipients survived beyond 30 minutes. One animal died at 43 minutes for unclear reasons, one died at 4 hours and one died at 6 hours because of bleeding complications at the anastomotic sites that could not be controlled, and three animals survived until they were euthanized at ≈8 hours. These empirical studies suggest that the benefit we observed in the NTG group at 30 minutes is likely to continue well beyond the 30-minute observation period.

Because it has been suggested that vasodilators enhance pulmonary preservation by lowering PVR during harvest,[2,25] resulting in more rapid and effective distribution of hypothermic preservation solution, we determined the flushing time required to deliver identical volumes of preservation solution at identical flushing pressure as a reflection of PVR during harvest. These experiments demonstrated that NTG did indeed lower PVR during flushing, resulting in more rapid preservation than in its absence (86.4±4.9 versus 192.7±9.6 seconds, $P<.01$ respectively; Fig 4). However, harvest vasodilation alone was insufficient to enhance pulmonary preservation, as hydralazine (0.02 μg/mL, a direct-acting vasodilator[26]) was even more effective at harvest pulmonary vasodilation than was NTG (flush time, 69.3±4.5 seconds; $P<.05$ versus NTG, $P<.005$ versus EC alone) but relatively ineffective at enhancing pulmonary preservation for transplantation (Figs 2 and 3). Grafts preserved with hydralazine (0.02 μg/mL) demonstrated poor function (reduced arterial oxygenation), poor PA blood flow, elevated PVR, and poor recipient survival (Figs 2 and 3).

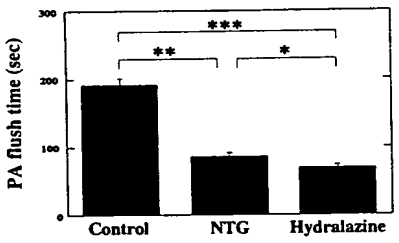

FIG 4. Bar graph showing the effects of vasodilators added to the preservation solution on pulmonary vasodilation during harvest. Effect of preservation solution on pulmonary artery (PA) flushing time during harvest of the lung is shown: n=6 for control (Euro-Collins solution [EC] alone), n=5 for EC plus nitroglycerin (NTG, 0.1 mg/mL), and n=6 for EC plus hydralazine (0.02 μg/mL). *$P<.05$, **$P<.01$, and ***$P<.005$.

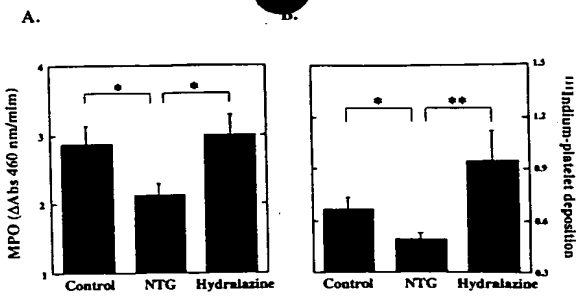

FIG 5. Bar graphs showing effects of nitroglycerin (NTG) or hydralazine added to the pulmonary preservation solution on graft neutrophil and platelet accumulation. A, Myeloperoxidase activity (MPO) was used to quantify neutrophil deposition (n=6 for each group). ΔAbs indicates change in absorbance. B, ^{111}In-labeled platelet accumulation, expressed as the ratio of graft radioactivity to blood radioactivity (n=6 for each group). *$P<.05$ and **$P<.01$.

These data suggested that the beneficial effects of NTG were not exclusively due to its actions as a vasodilator. Because NTG inhibits platelet aggregation[27] and neutrophil adherence to the reperfused coronary endothelium[5] and because neutrophil aggregation and platelet plugging have been implicated in the no-reflow phenomenon,[27,28] we evaluated the effects of NTG added to the preservation solution on graft neutrophil and platelet accumulation after reperfusion. NTG (0.1 mg/mL) added to the preservation solution was associated with a significant decline in both neutrophil and platelet accumulation in the reperfused grafts, as quantified by graft myeloperoxidase activity (Fig 5A) and ^{111}In-labeled platelet deposition (Fig 5B). In contrast, the addition of hydralazine to the pulmonary preservation solution did not alter the platelet or neutrophil deposition compared with the control solution (Fig 5). It is likely that these actions of NTG added to the pulmonary preservation solution are occurring within the confines of the transplanted lung and are not secondary to systemic effects, because measurements of bleeding times did not differ between animals transplanted with control or NTG-preserved lungs (Fig 6).

Discussion

Vascular endothelium plays a cardinal role in maintaining a homeostatic milieu both within blood vessels and the tissues they supply. NO serves as an important signaling molecule to reduce vasomotor tone of the subjacent vascular smooth muscle,[6] maintain endothelial barrier properties,[10] reduce platelet aggregation,[5] and inhibit neutrophil adherence to the endothelial surface.[9] After a period of ischemia, these physiological endothelial properties are perturbed, favoring vasoconstriction,

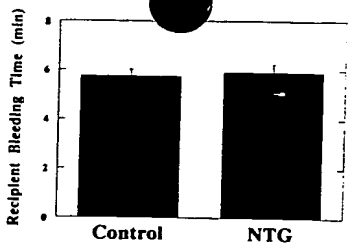

FIG 6. Bar graph showing the effect of nitroglycerin (NTG) added to the pulmonary preservation solution on systemic platelet function. Bleeding times (measured as the time required for hemostasis after uniform tail transection, as described in "Materials and Methods") were used as an index of platelet function and were recorded immediately after reperfusion of lungs preserved with supplemental NTG (0.1 mg/mL, n=7) versus lungs preserved with control solution (n=10).

thrombosis, and neutrophil adhesion, as the inflammatory response is activated. NO levels plummet after endothelial cell exposure to hypoxia and reoxygenation because of the rapid production of superoxide during reoxygenation.[5] Because the loss of available NO may contribute to endothelial dysfunction and pulmonary graft failure during the immediate posttransplant period, the experiments presented here confirmed the hypothesis that an NO donor such as NTG would enhance NO-related mechanisms of vascular homeostasis within the pulmonary graft. Furthermore, these experiments used hydralazine to demonstrate that vasodilation alone at the time of harvest is insufficient to protect the lungs but that reducing neutrophil and platelet sequestration into the reperfused graft is important.

NO produced within the lungs has important physiological functions,[17] mediated by increases in intracellular cGMP within target cells.[29] NTG is thought to act by way of intracellular *S*-nitrosothiol intermediates to directly stimulate guanylate cyclase or to release NO locally in effector cells[29-32] and has recently been shown to increase NO in expired air,[33] suggesting that NTG contributes to local levels of NO within the lungs. Other experiments in our laboratory demonstrate that supplementing NTG in the preservation solution augments tissue cGMP levels (unpublished radioimmunoassay data, 1994), suggesting that incorporating NTG into the pulmonary preservation solution is an effective means of delivery. The present set of experiments was designed to test whether NTG added to a pulmonary preservation solution might augment vascular homeostasis within the reperfused graft, thereby improving graft function and recipient survival. These experiments demonstrate that NTG is unequivocally effective in this regard, resulting in a marked stabilization of pulmonary hemodynamics and improved arterial oxygenation after transplantation. This is in contrast to hydralazine, which, although it is an effective vasodilator at the time of harvest, does not demonstrate the antineutrophil or antiplatelet effects that NTG does.

The preservation of solid organs for transplantation has improved considerably over recent years, largely because of improvements in preservation techniques that enhance parenchymal function of the transplanted organs. However, the lungs remain among the most problematic organs for transplantation, for reasons that are not fully understood. In our studies, the beneficial effects of NTG in the setting of lung transplantation were not limited to vasodilation but included reduced neutrophil and platelet accumulation and improved gas exchange. These data are consistent with the observation of others that inhibiting platelet[34] and neutrophil[20,35-37] accumulation is also important after ischemia and reperfusion. Of the many different preservation strategies described in the experimental literature, only donor prostaglandin administration has been used in clinical lung transplant centers. However, the use of prostaglandins to improve donor preservation has remained sporadic because their effectiveness is controversial.[2] Although prostaglandins per se were not tested in the present series of experiments, harvest vasodilation alone is insufficient to adequately preserve lungs, as demonstrated by our experiments in which an effective vasodilating dose of hydralazine during harvest failed to protect the lungs during reperfusion. Our data indicating that the vasodilator hydralazine is an ineffective pulmonary preservative are concordant with previously published data.[38] In contrast to hydralazine, prostaglandins have a theoretical advantage in that they may improve vascular homeostasis by enhancing levels of the intracellular second messenger cAMP,[39,40] not only promoting vasodilation but inhibiting neutrophil adhesion and platelet aggregation as well.[41,42] This hypothesis is currently the subject of further investigation in our laboratory.

The experiments presented here contribute to the growing body of evidence characterizing the detrimental role of neutrophils in pulmonary ischemia/reperfusion. Depletion of neutrophils from the perfusate[20,35-37] has been shown to improve the function of reperfused lungs. In the present study, we have shown that attenuation of neutrophil accumulation within the pulmonary graft by NTG supplementation paralleled improved graft function and recipient survival after orthotopic transplantation. It is not surprising that NTG may interfere with neutrophil accumulation during reperfusion, as NO has been shown to interfere with neutrophil/endothelial adhesion,[9] and local NO donors/analogues blunt myocardial injury and neutrophil accumulation during cardiac reperfusion.[43] This attenuated neutrophil infiltration might contribute to the beneficial effects of NTG, because recruited neutrophils release numerous toxic compounds, including superoxide anion, chloramine, hypochlorous acid, hydroxyl radical, and hydrogen peroxide, as well as lysosomal contents, such as elastase, the metalloproteases (collagenase and gelatinase), neutral proteases, and heparinase.[44]

The initial source of superoxide after reperfusion in the lungs is not clear, although endothelial cells themselves have been shown in vitro to rapidly generate superoxide after hypoxia and reoxygenation.[45] These initially formed oxygen radicals in the reperfusion milieu are potent neutrophil chemoattractants and activators[46] that may compound subsequent neutrophil accumulation/activation, resulting in rapid graft failure. Grafts preserved with NTG appear to have a reduction in tissue oxidant stress after transplantation, as measured by the presence of thiobarbituric acid reactive substances (data not shown), supporting the potential beneficial outcome of reducing graft neutrophil infiltration. The initial inhibition of neutrophil recruitment into the reperfused

graft by NTG may not only improve pulmonary parenchymal function but, by decreasing the reactive oxygen intermediate milieu, may result in greater local concentration of NO. As reactive oxygen intermediates induce prolonged expression of the neutrophil adherence molecule P-selectin on the endothelial surface, which mediates rapid neutrophil adhesion to the endothelium,[47] initial reductions in neutrophil accumulation may be magnified by attenuating the production of reactive oxygen intermediates, further reducing neutrophil adhesion and activation. In this manner, the beneficial vascular effects of NTG may be magnified by its ability to attenuate the early phases of neutrophil adhesion.

Reactive oxygen intermediates formed within lungs subjected to ischemia and reperfusion may rapidly combine with NO, forming highly toxic intermediates such as peroxynitrite and hydroxyl radical in the process.[19,48] This has caused reservations about the use of inhaled NO in the setting of pulmonary reperfusion. NTG may avoid this theoretical problem by directly activating guanylate cyclase via *S*-nitrosothiol intermediates.[29-32] Although agents designed to limit the formation of reactive oxygen intermediates[49,50] have been studied in pulmonary preservation, none are used routinely for clinical lung transplantation. In pilot studies of our own, superoxide dismutase (conjugated to polyethylene glycol to extend its half-life in the circulation) administered to the pulmonary transplant recipient before reperfusion failed to protect the lungs compared with the control solution. This may relate to the extremely rapid kinetics of the reaction between superoxide and NO, with a rate constant of 10^8 $(mol/L)^{-1} \cdot s^{-1}$, which effectively competes with the dismutation of superoxide,[19] or may relate to the relatively large size of the superoxide, limiting its accessibility to sites of superoxide formation.

Because the reaction of superoxide with NO leads to the formation of highly toxic peroxynitrite and hydroxyl radicals, we performed limited experiments in which we tested whether blocking endogenous NO synthesis might, in combination with NTG, enhance pulmonary preservation. In these experiments, we tested the effects of N^G-monomethyl-L-arginine (L-NMMA, a competitive inhibitor of NO synthesis) on graft preservation; addition of L-NMMA alone (5 μmol/L) to EC was associated with 100% recipient death (n=3), whereas L-NMMA plus supplemental NTG (0.1 mg/mL, n=3) was associated with 100% recipient survival. Further studies are currently under way to confirm whether inhibiting endogenous NO synthesis concomitant with the addition of supplemental NTG may have a pulmonary protective effect.

The studies presented here demonstrate that a drug (hydralazine) that merely causes vasodilation at the time of pulmonary harvest but lacks other important vascular effects (such as the ability to reduce neutrophil and platelet sequestration after reperfusion) does not protect the lungs after transplantation. In contrast, NTG, which is an effective harvest vasodilator but which also has potent antineutrophil and antiplatelet actions, can improve gas exchange, reduce pulmonary vascular resistance, improve graft blood flow, and improve recipient survival after lung transplantation. These studies emphasize the pluripotent benefits of augmenting an important endogenous signaling pathway (NO), which may be depressed after pulmonary reperfusion.

Acknowledgments

This study was supported in part by grants from the Cystic Fibrosis Foundation (Dr Pinsky) and a Grant-in-Aid from the American Heart Association (Dr Pinsky). Dr Oz is the recipient of an Irving Scholarship, and Dr Pinsky is a Clinician Scientist of the American Heart Association.

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